NOTE *Pathology*

A Canine Case of Primary Intra-Right Atrial Paraganglioma

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ABSTRACT. An 11-year-old mixed breed dog was presented with signs of anorexia. Radiographic and ultrasound examinations revealed a large mass in the heart, between the right atrium and the right ventricle. Upon gross inspection, a multilobulated tumor arising from the right atrial wall and occupying the right atrium was identified. Microscopical analysis demonstrated that this tumor consisted of neoplastic cells with granular cytoplasm, which were separated into nests by fine fibrovascular stroma and were negative for Grimelius's method. Immunohistochemical examinations revealed that the neoplastic cells expressed chromogranin A, synaptophysin and neuron specific enolase. Electron microscopy revealed that the cytoplasm of the neoplastic cells held secretory granules. Based on these pathological findings, the tumor was diagnosed as a paraganglioma. This report is a rare case of primary paraganglioma deriving from the right atrium and provides a detailed characterization of its morphological features.

KEY WORDS: canine, morphological diagnosis, paraganglioma, right atrium.

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Paragangliomas originate from extra-adrenal neural crest-derived endocrine organs, known as paraganglia, and are chromaffin-positive [3, 11]. Microscopically, the tumor cells are typically arranged in nests or packets, known as a Zellballen pattern, surrounded by sustentacular cells [3]. Immunohistochemically, they are positive for chromogranin, synaptophysin and neuron specific enolase (NSE). Ultrastructurally, both adrenalin- and noradrenalin-type granules are demonstrated in the cytoplasm of the neoplastic cells [3].

Primary neoplasms at the heart base are uncommon in domestic animals with the exception of hemangiosarcomas, arising from the right atrium, and aortic body tumors (chemodectomas) in dogs [4, 6]. Intracardiac paragangliomas are rare among mammals, including humans [2, 10, 14]. In this study, we describe a case of canine right atrial paraganglioma with detailed histopathological, immunohistochemical and ultrastructural findings.

An 11-year-old female mixed-breed dog was presented with depression and anorexia. On auscultation, a heart murmur was heard. Radiographic and ultrasound examinations revealed a large mass between the right atrium and the right ventricle with pleural and peritoneal effusions. Respiratory clinical signs and lethargy were not observed. The dog was euthanized after several appointments for symptomatic treatment. The clinician performed the necropsy and found cardiac enlargement of the right side, hepatic congestion and mild splenic atrophy. The heart, liver and spleen were fixed in 10% buffered formalin and submitted to the laboratory for histopathology.

A cardiac tumor (3.5 × 4.5 cm diameter) protruded from the right atrial wall and almost obstructed the right atrium (Fig. 1). The surface of the right atrial epicardium was intact, and no lesion was observed around the right and left atria, pericardium and aorta. The cut surface of the tumor displayed multilobular pattern with white to grey-tan color (Fig. 2). The submitted tissues, fixed in 10% buffered formalin, were routinely processed and embedded in paraffin wax. The tissue sections (3 μm) were stained with hematoxylin and eosin (HE), silver impregnation (Watanabe's method) and Grimelius's method. Immunohistochemical (IHC) analyses were performed using the polymer method with EnVision+ (Dako, Glostrup, Denmark). The primary antibodies used in these experiments were as follows: chromogranin A (Nichirei, Tokyo, Japan; prediluted), synaptophysin (Nichirei; prediluted), NSE (Dako; prediluted), S100 protein (Nichirei; prediluted), neurofilament protein (Dako; prediluted) and cytokeratin (Nichirei; prediluted). For transmission electron microscopy, the formalin-fixed tissue was post-fixed in 1% buffered osmium tetroxide and embedded in an Epoxy resin. Ultrathin sections (70 nm) were stained with uranyl acetate and lead citrate and examined with a transmission electron microscope (TEM) (H-7650, Hitachi, Tokyo, Japan).

Histological analysis revealed expansive growth of the neoplasm and fine fibrovascular stroma separating the neoplastic cells into variable sized trabeculae or nests (Zellballen pattern) (Fig. 3). The neoplastic cells were cuboidal to polyhedral in shape and eosinophilic and had finely granular cytoplasms. The nuclei were pale, round to oval and occasionally large in irregular shape (Fig. 3, inset). Mitotic figures were scattered throughout the tumor (0–4/10 high power fields). Each packet of cells was surrounded by reticulin fibers (Fig. 4). Grimelius's method was negative within the neoplastic cells. IHC analysis indicated that the

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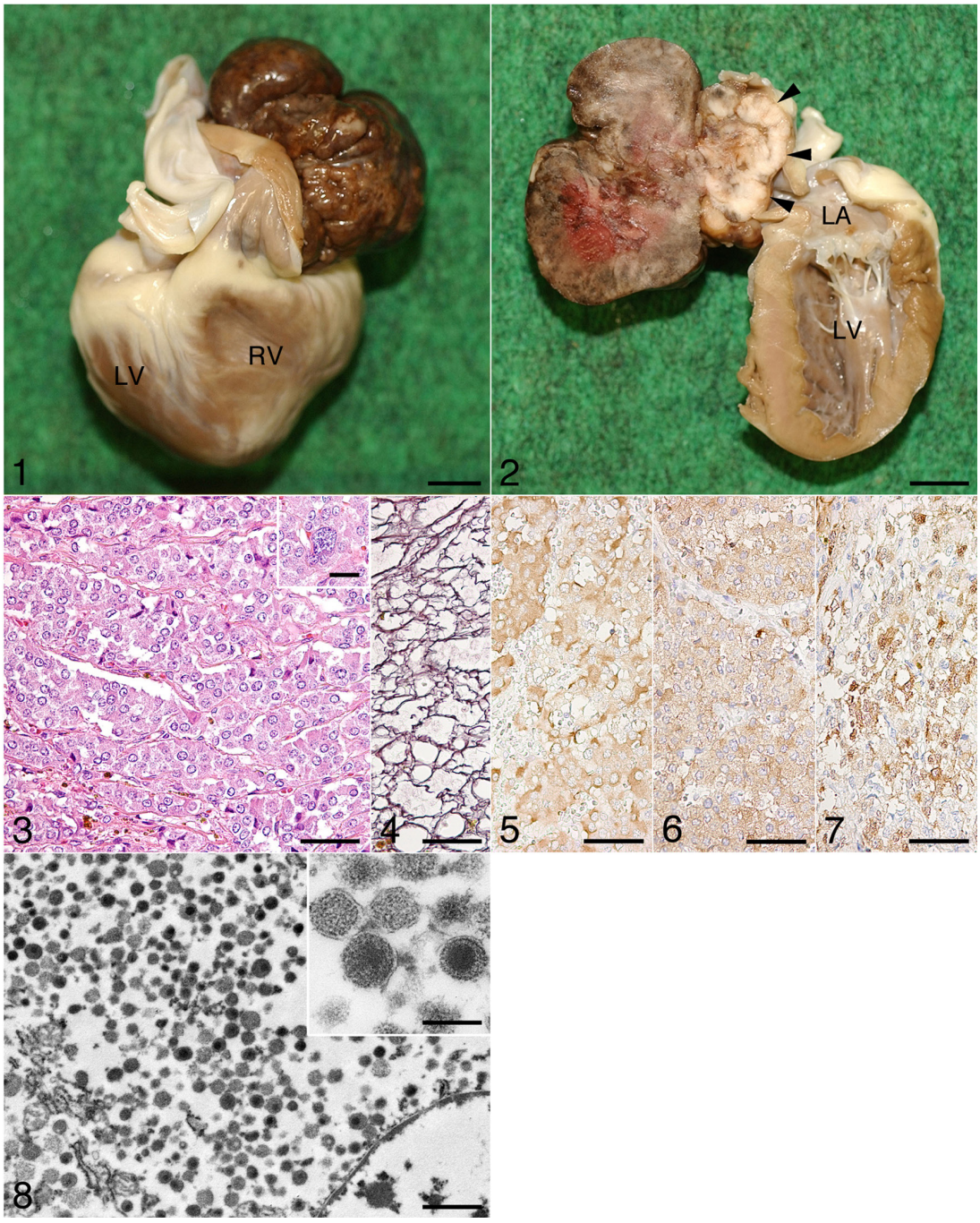

Fig. 1. Gross findings. The mass arises from the right atrial wall. LV: left ventricle; RV: right ventricle. Bar=1 cm.
Fig. 2. Cross section of the tumor. The cut surface displays a multinodular pattern with a lobulated texture that is white to gray-tan in color. Arrowheads: right atrial wall; LA: left atrium; LV: left ventricle. Bar=1 cm.
Fig. 3. Histological appearance. Each trabecula or nest of the neoplastic cells is divided by fine fibrovascular septa. HE. Bar=40 μm. Inset: the occasional neoplastic cells have giant nuclei. HE. Bar=20 μm.
Fig. 4. The nests of the neoplastic cells are divided into small components by reticulin fibers. Silver impregnation. Bar=40 μm.
Fig. 5. Expression of chromogranin A in the cytoplasm of the neoplastic cells. IHC. Bar=40 μm.
Fig. 6. Expression of synaptophysin in the cytoplasm of the neoplastic cells. IHC. Bar=40 μm.
Fig. 7. Expression of NSE in the cytoplasm of the neoplastic cells. IHC. Bar=40 μm.
Fig. 8. Ultrastructurally, the neoplastic cells contain many intracytoplasmic granules. TEM. Bar=1 μm. Inset: each granule is covered by limiting membrane with halo. TEM. Bar=300 nm.

neoplastic cells were positive for chromogranin A (Fig. 5), synaptophysin (Fig. 6) and NSE (Fig. 7); however, there was no immunoreactivity for the other antibodies. Ultrastructurally, the neoplastic cells contained a large number of granules measuring approximately 160–250 nm in diameter (Fig. 8). The cores displayed a granular texture and occasionally contained a highly electron dense inner region (Fig. 8, inset). These granules were covered by a limiting membrane with a narrow halo. The liver and spleen did not have any tumor-related lesions and displayed nodular hyperplasia and mild extramedullary hematopoiesis, respectively.

Based on the pathological findings, this case was consistent with a tumor of neuroendocrine origin. Neuroendocrine tumors are categorized as endodermal (epithelial) or neuroectodermal in origin [15]. Endodermal tumors arise in the pituitary gland, parathyroid gland, C-cells of the thyroid gland, pancreatic islets or the diffuse endocrine system of luminal organs, such as the gastrointestinal tract (carcinoid), whereas neuroectodermal tumors arise from the adrenal medulla and from paraganglia [11, 15]. Neuroendocrine tumors are characterized by the presence of intracytoplasmic hormonal secretory granules, which are visualized using argyrophil reaction, such as Grimelius's methods [15]. Immunohistochemistry using antibodies against chromogranin is also used to identify neuroendocrine tumors [15]. The paraganglia can be sympathetic or parasympathetic in nature [12]. The adrenal medulla is a type of sympathetic paraganglion, whereas, typical parasympathetic paraganglia include chemoreceptors (the carotid/aortic body), the vagal body and cells associated with the thoracic, intra-abdominal and retroperitoneal ganglia [11–13]. In parasympathetic paraganglia, paragangliomas are defined as arising from chromaffin cells [11]. Paragangliomas can be subdivided to functional or non-functional. Chemoreceptor tumors are non-functional [4, 13].

Due to the limited clinical findings in the present case, it was not possible to determine whether the tumor was functional or non-functional. This case was not reactive for Grimelius's method, but it was positive for neuroendocrine markers on IHC. Although the cause was unknown, several human cases of neuroendocrine neoplasms have been diagnosed by ultrastructural or immunohistochemical evidences even though those cases were negative for argyrophil reaction [7, 9]. Ultrastructural figures of the intracytoplasmic granules in the present case displayed a slightly different texture from the ones observed in paragangliomas. The electron density of secretory granules varies considerably with the fixative used [4]. The heart was received intact at the laboratory three days after necropsy in insufficient fixative. Inappropriate fixation of the submitted organ may have influenced the ultrastructural morphology of intracytoplasmic granules. S100 protein-positive cells were not detected in this tumor. In general, nests of the neoplastic cells in paragangliomas are surrounded by sustentacular cells, which are positive for S100 protein [13]. The sustentacular cells in two cases of human metastatic paragangliomas, however, were negative for S100 protein [1, 8]. No S100-positive cells in this tumor may also result from inadequate fixation, or the sustentacular cells may not be involved in this case.

In the present case, the tumor developed from the right atrial wall and protruded into the lumen without exophytic growth and involving other tissues. The aortic body is normally located in the periadventitial tissue of the ascending aorta and pulmonary trunk within the pericardial sac [4]. Therefore, most of the aortic body chemodectomas are located on the epicardial surface of the heart base or atria [3] and often involve aorta and/or atrial, interatrial septum [4]. Paraganglial cells are generally located within the atria along the atrioventricular sulcus and near the roots of the great vessels [3, 5]. Therefore, based on the histopathological, immunohistochemical and ultrastructural findings, the case was diagnosed as right atrial paraganglioma.

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